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PATENT ABSTRACTS OF JAPAN

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(54) MODEL ANIMAL FOR ALZHEIMER'S DISEASE

(57)Abstract:
PROBLEM TO BE SOLVED: To obtain the subject new model animal comprising a mammalian or the like obtained by generating an individual from a totipotent cell containing human presenilin 2 gene or the like, having the gene in the chromosomes of body cells, and useful for analyzing the states of Alzheimer's diseases, developing medicines therefor, and the like.
SOLUTION: This model animal for Alzheimer's diseases comprises a mammalian obtained by generating an individual from a totipotent cell containing human presenilin 2 gene or its variant transduced thereinto or the progeny of the mammalian, wherein the transduced gene is held in the chromosomes of body cells. The model animal is used for analyzing the states of Alzheimer's diseases, developing medicines for treating the diseases, and the like. The model animal for the Alzheimer's diseases is obtained by extracting all RNAs from the brain of a healthy old person, preparing cDNAs from the all RNAs, obtaining a wild type presenilin 2 cDNA by a PCR amplification method or preparing a variant cDNA in which a mutation is partially introduced by a PCR mutation-inducing method, inserting the product into a vector, injecting the vector into a mouse embryo, injecting the embryo into a provisional parent oviduct, allowing to generate an individual, and subsequently making to bear the individual.

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CLAIMS

[Claim(s)]
[Claim 1] The Alzheimer disease model animal which are the mammalian obtained by carrying out the ontogeny of the totipotency cell which introduced Homo sapiens presenilin 2 gene or its mutant alle, and its descendant animal, and is characterized by holding the above-mentioned introductory gene in a somatic cell chromosome.
[Claim 2] The Alzheimer disease model animal of claim 1 whose mammalian is a mouse.
[Claim 3] The Alzheimer disease model animal of claims 1 or 2 whose Homo sapiens presenilin 2 genes are cDNA prepared from RNA of the genome gene concerned.
[Claim 4] The Alzheimer disease model animal of claims 1 or 2 whose mutants alle of Homo sapiens presenilin 2 are the variation cDNA which the 141st asparagine residue in cDNA prepared from RNA of the genome gene concerned permuted by isoleucine residue.
[Claim 5] The Alzheimer disease model animal of claims 1 or 2 whose mutants alle of Homo sapiens presenilin 2 are the variation cDNA which the 239th methionine residue in cDNA prepared from RNA of the genome gene concerned permuted by valine residue.
[Claim 6] The cell isolated from one animal of claims 1-5.
[Claim 7] The test method which medicates one animal of claims 1-5 with the matter with the misgiving leading to an Alzheimer disease, and specifies an Alzheimer disease causative agent as it by making the volume of the amyloid beta protein in an animal cell into an index.
[Claim 8] The test method which cultivates by the culture medium containing the matter which has the misgiving leading to an Alzheimer disease in the cell of claim 6, and specifies an Alzheimer disease causative agent by making the amyloid beta protein volume of a cell into an index.
[Claim 9] The test method which medicates with the candidate remedy of an Alzheimer disease this animal that prescribed one of the animals or Alzheimer disease causative agents of claims 1-5 for the patient, and specifies an Alzheimer disease remedy as it by making the volume of the amyloid beta protein in an animal cell into an index.
[Claim 10] The test method which adds the candidate remedy of an Alzheimer disease, cultivates a cell, and specifies an Alzheimer disease remedy by making the amyloid beta protein volume of a cell into an index in the culture medium of this cell cultivated by the inside of the culture culture medium of the cell of claim 6, or the culture medium containing an Alzheimer disease causative agent.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] Invention of this application relates to an Alzheimer disease model animal. In more detail, invention of this application is a transgenic animal which holds Homo sapiens pre serine 2 gene (it may be hereafter indicated as PS2) which is one of the genes of cause of an Alzheimer disease in a somatic cell chromosome, and relates to the cell culture of a model animal useful to development of a cure in a cause break through and its diagnostics list of an Alzheimer disease, and this animal, and the various test methods using these.
[0002]
[Description of the Prior Art] The symptoms of an Alzheimer disease (Alzheimer's Disease : AD) is shown after the middle age, and it is a progressive dementia disease which produces lowering of immediate memory and mneme, a personality disorder, etc. between short period of times, and is characterized [big] by existence of the senile plaque within a brain, and the intracellular neurofibrillary change. It is the important disease which can be said also as the nucleus of old age dementia together with cerebrovascular dementia, and in our country, 40 - 60% is called about 1/3 of a dementia patient, and is called AD in the U.S.
[0003] Moreover, about ten percent of AD is familial [which shows autosomal dominant inheritance / AD], and the variation of an amyloid beta protein precursor (betaAPP) gene is identified as one of the gene of cause of the in 1991 (Nature, 349:704-706, 1991). The variation of this betaAPP gene is participating in AD onset of family line which carries out a chain to the No. 21 chromosome, and the gene variation (Science 269:973-977, 1995) of presenilin 2 (PS2) is identified after that by the family line in which the gene variation (Nature375:754-760, 1995) of presenilin 1 (PS1) carries out a chain to the No. 1 chromosome in the family line which carries out a chain to the No. 14 chromosome.
[0004] and about betaAPP, creation of the transgenic mouse which discovers this variation protein tries — having — 1995 — Games ** — beta transgenic mouse reproducing symptoms change of AD is reported (Nature 373:523-527, 1995), and the analysis of the symptoms of AD using such a model animal and development of a remedy agent are expected.
[0005]
[Problem(s) to be Solved by the Invention] however — AD — the above — a passage — plurality — a gene — unusual — therefore — causing — having — a multifactorial disorder — it is — things — an idea — ** — **** — the analysis of the cause of a disease of AD, and symptoms and the development of a remedy agent only by the transgenic mouse related to betaAPP from things sake — enough — **** — it cannot say.
[0006] Although look like [the die length of a C terminal] especially the amyloid beta protein (Abeta) that is the major component of a senile plaque is divided into two kinds, Abeta42 and Abeta40 Among these, it is known that Abeta42 has played the role important for formation of a senile plaque. (For example) Neuron 13:45-53 and 1994; Ann.Neurol.37 : 294-299, 1995; Biochemistry 32: 4693-4697, 1993; Science 264: 1336-1340, 1994; Neurology 48: 741-745, 1997. And it is also known by this production of Abeta42 that the variation of PS1 and PS2 gene is participating in division into equal parts (Nature Med.2:864-870, 1996; Nature 383:710-713, 1996;

Neuron 17:1005-1013, 1996; Nature Med.3:67-72, 1996).
[0007] Therefore, for the symptoms analysis of AD, development of a remedy agent, etc., the transgenic animal which introduced the gene PS 1 related to production of Abeta42 which plays a role important for formation of the senile plaque which is the description of AD, or PS2 gene is indispensable. Invention of this application is made in view of the situation as above, and aims at offering the transgenic animal which holds Homo sapiens PS2 gene or its mutant alle as a new AD model animal.
[0008] Moreover, invention of this application is required for the purpose of offering the various test methods using the cell isolated from this model animal or animal.
[0009]
[Means for Solving the Problem] Invention of this application is the mammalian obtained by carrying out the ontogeny of the totipotency cell which introduced Homo sapiens presenilin 2 gene or its mutant alle as what solves the above-mentioned technical problem, and its descendant animal, and offers the Alzheimer disease model animal characterized by holding the above-mentioned introductory gene in a somatic cell chromosome.
[0010] In the model animal of this invention, the above-mentioned mammalian is setting to be a mouse to one of the desirable modes. Moreover, it requires that it is the variation cDNA which the variation cDNA which the 141st asparagine residue in cDNA which the mutant alle of that Homo sapiens presenilin 2 gene is cDNA prepared from RNA of the genome gene concerned and Homo sapiens presenilin 2 prepared from RNA of the genome gene concerned permuted by isoleucine residue, or the 239th methionine residue permuted by valine residue also as another desirable mode.
[0011] Invention of this application offers the cell isolated from the above-mentioned model animal again. Invention of this application offers the test method which specifies an Alzheimer disease causative agent or a remedy further again using the above-mentioned model animal or a cell. Hereafter, the gestalt of operation is explained in detail about each above-mentioned invention.
[0012]
[Embodiment of the Invention] AD model animals of this invention are the mammalian obtained by carrying out the ontogeny of the totipotency cell which introduced Homo sapiens PS2 gene or its mutant alle, and its descendant animal, and are transgenic animals characterized by holding the above-mentioned introductory gene in a somatic cell chromosome.
[0013] Homo sapiens PS2 gene (wild type PS 2) which is one of the introductory genes can create a cDNA library from RNA extracted from the human brain, and can obtain it by screening a library using the oligonucleotide probe compounded based on PS2 well-known array (Nature 376:775-778, 1995; Science 269:970-977, and 1995). Moreover, the target cDNA can be obtained also by the PCR method which makes a primer the synthetic oligonucleotide equivalent to the ends of PS2 well-known array.
[0014] Homo sapiens PS2 mutant alle (variant PS 2) can be easily created by carrying out variation induction by the well-known approach to cDNA of the wild type PS 2 obtained by the approach as above-mentioned. That is, a variant PS 2 is two kinds, the variation (N141I) which the 141st asparagine residue of wild type PS2cDNA permuted by isoleucine residue, and the variation (M239V) which the 239th methionine residue permuted by valine residue, (Neuron 18:687-690, 1997), and which variant PS 2 can be targetted for it by this invention.
[0015] Moreover, the promotor array and enhancer sequence for controlling the manifestation are connected with these introductory genes. About these arrays, there is especially no limit and it can be used, combining the array usually used suitably. However, in order to make an introductory gene discover specifically in a brain, beta-actin promotor's etc. activity is desirable.
[0016] According to a well-known approach (for example, Pro.Natl.Acad.Sci.USA 77:7380-7384, 1980), creation of a transgenic animal introduces the above-mentioned introductory gene into the totipotency cell of mammalian, generates this cell to an individual, and can be created by sorting out the individual by which the introductory gene was incorporated into the genome of a somatic cell. Although it is possible as mammalian to be aimed technical at all animal species, especially the inbred strain is created in large numbers, and the mouse with which techniques,

such as culture of a fertilized egg and in vitro fertilization, are moreover ready is the optimal. In the case of a mouse, it can be aimed at a cultured cell like the embryonic stem cell which has pluripotency besides a fertilized egg or an early embryo as a totipotency cell which introduces a gene. moreover, the case where the production effectiveness of a transgenic animal individual and the transmission efficiency of the introductory gene to the next generation are taken into consideration although the well-known electrostatic pulse method, the liposome method, the calcium phosphate method, etc. could be used as a transgenics method to such a cultured cell -- physical impregnation (microinjection) of the DNA solution to a fertilized egg — law is desirable.

[0017] The totipotency cell which put the gene is transplanted to the oviduct of assumed parents next, after making a foster parent attach and breed to an individual the animal occurred and born, extracts DNA from bodily [some] (for example, tail head), and checks existence of an introductory gene by Southern blot analysis, PCR assay, etc. The individual by which existence of an introductory gene was checked is transmitted to the founder (Founder), then an introductory gene by 50% of the descendant, and the animal which included stably the wild type PS 2 or the variant PS 2 in a part of chromosome can be created efficiently.

[0018] Thus, in the case of the animal which holds a variant PS 2, the created transgenic animal becomes with the optimal model animal for screening of the drugs with which AD curative effect is expected by the production depressant action or disintegration in order to overproduce Abeta42, as shown also in the example which carries out a postscript. Moreover, the transgenic animal which holds a wild type PS 2 is useful as a model animal for investigating the cause of a disease or natural onset process of AD. That is, since there are few volumes of Abeta42 compared with variant PS2 animal, wild type PS2 animal can specify AD causative agent by prescribing the matter with the misgiving leading to AD for the patient, and seeing extent of the increment in the volume. Or by AD causative agent, it can process beforehand and, subsequently to screening of AD remedy agent, can also use.

[0019] Since an introductory gene is held in all somatic cells, as for the transgenic animal offered by this invention, the cell isolated from this animal individual also produces Homo sapiens Abeta42 to each further again. For this reason, screening of AD causative agent or a remedy agent can be performed like the case of the above-mentioned animal individual using the culture system of these cells. Especially a cell culture system such is useful to primary screening of a causative agent or drugs as an animal experiment alternative.

[0020] Hereafter, an example is shown, and about this invention, further, although explained concretely, this invention is not a detail and the thing limited to the following examples.

[0021]

[Example]

(1) creation of a transgenic mouse — according to the well-known approach (Anal.Biochem.162:120-129, 1987), all RNA was extracted from a healthy old man's brain, reverse transcription was carried out by the well-known approach (J. Neurochem.67:1235-1244, 1996), and cDNA was created. Subsequently, cDNA (481-bp) of a wild type PS 2 was prepared by PCR magnification from these cDNA(s). As an PCR primer, the synthetic oligonucleotide (forward primer) of the array number 1 and the synthetic oligonucleotide (reverse primer) of the array number 2 were used. Moreover, PCR conditions were made into x(for 3 minutes: for 1 minute: 94 degrees C 55 degrees C 72 degrees C for 4 minutes) 35 cycle.

[0022] As cDNA of a variant PS 2, N141I which permuted the 141st arginine residue by isoleucine residue by the PCR variation creeping method of adjustment was created. Subsequently to expression vector pCAGGS (Gene 108: 200 192- 1991) these cDNA(s) were inserted, analyzed the base sequence by the DNA sequencer, and checked that they were wild type PS2cDNA and variant PS2cDNA (N141I), respectively.

[0023] Next, to the insertion of the recombination vector pCAGGS, insertion association of a CMV-IE enhancer sequence, a fowl beta actin promotor, and the rabbit beta globin polyadenylation signal was carried out, and the introductory gene was built. After it started this introductory gene from the vector and gel electrophoresis refined, it poured into the mouse germ by the microinjection method. The mouse germ was obtained by mating with BL/C57 6(B6) J, or

DBF1 (female) and B6N (male). a transgenics germ — a law — transplanted to the oviduct of assumed parents according to the method, and you made it generate to an individual, and made it born

[0024] DNA was extracted from the tail of the obtained mouse individual, and the PCR assay which makes a primer the oligonucleotide compounded based on the ends array of an introductory gene fragment sorted out the first transgenic mouse. Consequently, the first transgenic mouse which holds wild type PS2cDNA, and the one first transgenic mouse which holds variant PS2cDNA were obtained at a time, respectively. Backcross of these first transgenic mice was carried out to C57BL/6J, and two lines (W1 and W2) of the transgenic mouse which holds Homo sapiens wild type PS2cDNA, and two lines (M1 and M2) which hold Homo sapiens variant PS2cDNA were created.

(2) extent of the introductory gene expression within the brain of the examination transgenic mouse of the gene expression of a transgenic mouse -- reverse transcriptase-PCR (RT-PCR) -- it investigated by law at the event of eight-month age. Consequently, although the amount of manifestations of internality mouse PS2mRNA of a wild type mouse was detectable, by four transgenic mice, buildup of the amount of manifestations of introductory PS2mRNA was checked by each to being very small. Moreover, compared with the wild type mouse, it was 15 times (W1) the amount of manifestations of this from 5 times (M2). Moreover, W2 and M1 line had the almost same manifestation level of introductory PS2mRNA. In addition, the existence of an introductory gene had not affected the mouse PS 1 of internality, or the level of PS2mRNA.

[0025] From the above result, it was checked that the obtained transgenic mouse is overproducing the Homo sapiens wild type PS 2 and the variant PS 2 within a brain, respectively.

(3) Abeta40 and Abeta42 level in the brain soluble fraction in the event of the 2, 5, and eight-month age of Abeta40 [within the brain of a transgenic mouse], and the examination transgenic mice W2 and M1 of the level of Abeta42 -- immunoassay (EIA) — it investigated by law. A result is as having been shown in a table 1.

[0026] It was checked that Homo sapiens variant PS2 transgenic mouse (M1) has many Abeta42 within a brain amounts compared with control (wild-type mouse: Non-Tg) and Homo sapiens wild type PS2 transgenic mouse (W2) so that clearly also from the result of this table 1. Abeta42 level in M1 increased notably according to aging to especially Abeta42 level in W2 increasing slightly after five-month age. From these things, increasing Abeta42 amount in connection with aging was checked in the Homo sapiens variant PS2 transgenic-mouse brain.

[0027]

[A table 1]

Tg line	Age(months)	Aβ40(pmol/g)	Aβ42(pmol/g)	Aβ(pmol/g)	Aβ42/Aβ40
Non-Tg	2	< 0.040	0.043	< 0.083	-
		< 0.040	0.128	< 0.168	-
		< 0.040	0.119	< 0.159	-
		< 0.040	0.163	< 0.203	-
		< 0.040	0.075	< 0.115	-
	Mean ± s. d.	-	0.106 ± 0.047	-	-
	5	0.440	0.240	0.680	0.55
		0.420	0.240	0.660	0.57
		0.525	0.295	0.820	0.56
		0.375	0.205	0.580	0.55
		0.335	0.250	0.585	0.75
		0.465	0.275	0.740	0.59
	Mean ± s. d.	0.427 ± 0.067	0.251 ± 0.031	0.678 ± 0.092	0.595 ± 0.077
	8	0.392	0.565	0.957	1.44
		0.329	0.434	0.762	1.32
		0.423	0.316	0.738	0.75
		0.361	0.218	0.578	0.60
		0.446	0.254	0.700	0.57
		0.385	0.347	0.732	0.90
		0.361	0.284	0.645	0.79
		0.378	0.212	0.590	0.56
		0.343	0.248	0.590	0.72
	Mean ± s. d.	0.379 ± 0.037	0.320 ± 0.116	0.699 ± 0.120	0.850 ± 0.322
W2	2	< 0.040	0.097	< 0.137	-
		< 0.040	0.111	< 0.151	-
		< 0.040	0.093	< 0.133	-
		< 0.040	0.111	< 0.151	-
	Mean ± s. d.	-	0.103 ± 0.009	-	-
	5	0.125	0.115	0.240	0.92
		0.230	0.130	0.360	0.57
		0.100	0.082	0.182	0.83
		0.070	0.082	0.152	1.18
		0.055	0.068	0.122	1.23
	Mean ± s. d.	0.116 ± 0.069	0.096 ± 0.026	0.211 ± 0.094	0.946 ± 0.270
	8	0.202	0.176	0.378	0.87
		0.117	0.255	0.372	2.19
		0.206	0.317	0.522	1.54
		0.051	0.128	0.179	2.51
	Mean ± s. d.	0.144 ± 0.074	0.219 ± 0.084	0.363 ± 0.141	1.778 ± 0.727
M1	2	< 0.040	0.205	< 0.245	-
		< 0.040	0.201	< 0.241	-
		< 0.040	0.209	< 0.249	-
		< 0.040	0.214	< 0.254	-
		< 0.040	0.264	< 0.304	-
	Mean ± s. d.	-	0.219 ± 0.026	-	-
	5	0.430	0.350	0.780	0.81
		0.330	0.295	0.625	0.89
		0.305	0.315	0.620	1.03
		0.320	0.380	0.700	1.19
	Mean ± s. d.	0.346 ± 0.057	0.335 ± 0.038	0.681 ±0.075	0.980 ± 0.167
	8	0.198	0.465	0.663	2.35
		0.131	0.421	0.552	3.21
		0.135	0.406	0.542	3.01
		0.203	0.480	0.682	2.36
		0.092	0.317	0.409	3.45
	Mean ± s. d.	0.152 ± 0.048	0.418 ± 0.064	0.570 ± 0.110	2.876 ± 0.500

[0028]
[Effect of the Invention] The transgenic animal which holds Homo sapiens PS2 gene or its mutant alle is offered by this invention as explained in detail above. Development of the remedy agent etc. is promoted by these animals at the cause of a disease of AD and symptoms analysis, and a list.
[0029]

[Layout Table]
array number: -- die-length [of one array]: -- mold [of 30 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array Synthetic DNA array CCCGCCGGAA TTCCAGAGGC AGGGCTATGC 30 array number: -- die-length [of two arrays]: -- mold [of 30 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array Synthetic DNA array CCCGCCGAGA TTCAGATGTA GAGCTGATGG 30

[Translation done.]